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Artificial Riboswitch to Modulate HCV Infection

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Hepatitis C virus (HCV) is the main etiological agent causing chronic hepatitis, liver cirrhosis and, in some instances, hepatocellular carcinoma [1], Although HCV affects more than 3% of the world population, specific and efficient anti-HCV therapy and anti-HCV vaccine have not yet been developed.

HCV harbors a single and positive-stranded RNA genome of about 9,600 nucleotides in length encoding a polyprotein of about 3010 amino acids [2]. The polyprotein is co- or post-translationally processed into at least 10 mature structural and nonstructural proteins (C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) by cellular and viral proteases [2, 3].

HCV NS5B contains RNA-dependent RNA polymerase activity [4], which is important for the synthesis of negative-strand and genomic viral RNA during the HCV replication. Therefore, HCV NS5B is considered crucial for the viral proliferation, and hence, is a primary target molecule for the development of antiviral drugs [5].

Characteristics of RNA which can adopt complicated but stable structures to bind target molecules with high specificity and high affinity, encode amplificable genetic information, and can be chemically synthesized in a large amount render RNA a potentially very useful diagnostic and/or therapeutic compounds [6, 7]. Such short RNA ligands, termed RNA aptamers, have been selected from a combinatorial RNA library to bind a wide variety of molecules using *in vitro* iterative selection techniques, called systematic evolution of ligands by exponential enrichment (SELEX) [8, 9]. Several aptamers have been successfully validated in animal disease models [10-12], and some of them are now in therapeutically clinical development stage [13]. Noticeably, the US FDA recently approved RNA aptamer against vascular endothelial growth factor (VEGF), called pegaptanib sodium (Macugen), for the therapy of all types of neovascular age-related macular degeneration [14], which represents a great therapeutic potential of the RNA aptamers.

Here, I will describe our efforts at the development of an intracellularly antiviral inhibition strategy using RNA aptamers as decoys. We employed an RNA combinatorial library and isolated and

characterized RNA aptamers for the HCV NS5B replicase essential for the HCV multiplication. These aptamers very avidly and specifically bound the target proteins, and moreover acted as potent decoys to competitively inhibit biochemical activity of the replicase. Specific interaction between the aptamers and intracellular target protein was confirmed by Maldi-Tof analysis. Importantly, cytoplasmic expression of such aptamers efficiently impeded HCV subreplicon replication in human liver cells through interaction of the aptamer with the target NS5B protein in the cells. Moreover, we isolated high-affinity nuclease-resistant RNA aptamers with 2'-fluoro pyrimidines against the HCV NS5B. Direct transfection of these aptamers also showed efficient suppression of HCV replication in cells. These aptamers could be truncated up to chemically synthesizable 29 nt without compromising affinity and bioactivity. Therefore, identified aptamers could be applied to viral therapy through intracellular expression of the aptamer-encoding vector or direct inoculation into cells of their chemically synthesized forms.

Furthermore, we developed HCV-targeting hammerhead ribozyme which activity is allosterically regulated by NS5B RNA replicase. To this effect, we constucted random ribozyme library pool, which is consisted of sequence of the pre-selected RNA aptamer specific to NS5B, random 10 mer communication module sequence that can transfer structural transition for inducing ribozyme activity upon binding the NS5B protein to the aptamer, and sequence of HCV-targeting hammerhead ribozyme.

In vitro selection technology was performed to identify the most active communication module sequence which can induce ribozyme activity depending on the presence of NS5B protein. The ribozyme was inactive either in the absence of any proteins or in the presence of control bovine serum albumin. In sharp contrast, the selected ribozymes can induce RNA cleavage activity when incubated with the HCV NS5B protein with a rate constant (k_{min}) of ~0.15 min^{-1}. We employed 3'-RACE and RNase mapping analysis to find cleavage site and structural transition phenomena when HCV NS5B protein was administrated. Based on this study, we could develop a variety of allosteric ribozymes which activity can be induced or suppressed by combining protein-specific aptamer and ribozyme through communication module which sequence can be identified using *in vitro* selection technology.

These allosteric ribozymes will be more specific and effective as anti-HCV regimens because they can squelch target HCV proteins through aptamer binding to the proteins, and simultaneously, they can allosterically induce ribozyme activity only in HCV-infected cells expressing the target proteins. The controlled ribozyme could be function as riboswitch to turn off HCV replication through trans-cleaving target HCV RNA or releasing anti-HCV antisense molecules. These allosteric ribozymes can be applied as lead compounds for specific and effective anti-HCV agents, tools for highthroughput screening to isolate lead chemicals for HCV therapeutics, and biosensor probes for HCV diagnosis.

Keywords: aptamer, HCV, riboswitch, allosteric ribozyme

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